CLAIMS

1/ An in vitro diagnostic method of pathologies associated with gene rearrangement, wherein a patient's DNA or cDNA is subjected to a step of anchored PCR, characterized in that it comprises, in combination,

- one or several steps of asymmetrical PCR, carried out in a non specific manner, of gene rearrangements, by using a single pair of primers consisting in one primer specific of the nucleotidic sequence corresponding to gene liable to be involved in a fusion gene and one random primer, and

- a detection step of the PCR products, carried out by means of markers specific of gene rearrangements, in order to only detect the genes involved in a rearrangement, in their whole.

2/ The method according to claim 1, characterized in that the primers used in the amplification step include 25 to 40 nucleotides and Tm is of 75 to 85°C.

3/ The method according to claim 1 ~~or 2,~~ characterized in that the PCR products are marked in view of the detection step and are denatured, then put into contact with nucleotidic sequences specific of the nucleotidic sequences of the fusion partners.

4/ The method according to claim 3, characterized in that the probes are covalently secured on a support.

5/ The method according to claim 1 ~~or 2,~~

characterized in that the PCR products are denaturated and contacted with probes marked in solution.

6/ The method according to ~~any one of~~ claims 1 ~~to 5~~, characterized in that to obtain cDNA, sequences including a cassette of 40 to 60 nucleotides and 10 to 20 T patterns on one end or a random repeat of nucleotide pattern, with Tm of 80 to 90°C are used as primers.

7/ The method according to ~~any one of~~ claims 1 ~~to 6~~, characterized in that it comprises the following steps :

- the genome DNA, or RNA, extracted from the sample cells under investigation, are subjected to the action of a compound capable of cleaving or inhibiting specifically the DNA or RNA of the gene ,the fusion of which is under investigation
- the PCR steps, are performed and the products so obtained are allowed to react first with two specific probes of the gene under investigation one upstream and one downstream and next, using probes prepared from known partner genes,

where a positive detection on the upstream probe and a negative detection on downstream probe in the first case, evidencing a rearrangement of the relevant gene, and a negative detection in the second case evidencing that no known fusion product was detected or, alternatively,

- the PCR products are allowed to react with a plurality of probes secured on a miniaturized support, the probe PCR products, specific hybridization highlighting the

partner of the fusion gene.

8/ The method according to ~~any one of the~~ claims 1 ~~to 7~~, characterized in that it is applied to the detection of translocations involving the MLL gene, and includes

- the RT synthesis of a cDNA pool from the RNA extracted from the sample under investigation, using primers which include a cassette of 30 to 35 nucleotides complemented by a sequence of 6 or 9 random nucleotide patterns,
- the performance of an anchored PCR is completed using a primer located on the MLL' exon 5 as specific sense primer, optionally followed by a second amplification cycle, using an internal sens primer with respect to the first one, where the random primer is the same on each cycle and complementary to the oligonucleotide cassette used in the RT step.

9/ A method according to claim 8, characterized in that the detection of fusion transcripts, if any, includes putting :

- a specific probes of known MLL fusion partners, into contact with denatured PCR products marked by digoxygenine during amplification, in such conditions as will allow hybridization to occur where complimentary bases are present,
- resulting products into contact with anti-digoxygenine antibodies, such antibodies being coupled with an enzyme, capable of reacting with its substrate by releasing a colored product which can be detected if the antibodies should be bonded to PCR products, and then

- the probe/PCR product reactive mixture into contact with the enzyme substrate, and the product so formed if any, can be detected.

10/ A method according to claim 8 ~~or 9~~, characterized in that to detect new MLL-partner gene associations, total RNAs are subjected to the action of ribozymes MLL- gene specific before RT-PCR, then the amplification products are allowed to react first with a probe corresponding to MLL exon 5 sequence on the 3' end of the primer used, then with a second MLL gene-specific probe located between the break points and ribozymes action site, and finally with known partner probes.

11/ Application of the method according to ~~any one of the~~ claims 1 ~~to 10~~, to leukemia diagnostic.

12/ Application of the method according to ~~any one of the~~ claims 1 ~~to 10~~, to solid tumor diagnostic, such as Ewing tumors.

13/ Diagnostic kits to implement the method according to ~~any one of the~~ claims 1 ~~à 10~~, characterized in that such kits include the necessary reagents to perform the PCR and detection test, and as the case may be, the reverse transcription and/or reaction with agents capable of inhibiting or cleaving the gene, such kits further including primers for the foregoing various reactions and, preferably, the suitable solvents or buffers.

14/ Kits according to claim 13, characterized in that they comprise agents capable of cleaving or blocking the gene of the polypeptidic nucleic acids or of

the ribozymes.

15/ Kits according to claim 13 ~~or 14,~~ characterized in that they include oligonucleotide probes to perform the hybridations at the detection step, such probes being secured on a medium such as a multiwell plate, such as obtained by coupling a reagent on one of their ends with a reagent on the pate, for example by coupling biotin on their 5' end on streptavidine covering the bottom of a micro plate wells, or under still another embodiment, such oligonucleotide probes are secured on a miniature medium.

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ADD D13